clinical signs of Newcastle disease during the observation period, the Master Seed Virus is unsatisfactory.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(4) of this section.

(6) A strain identity test acceptable to Animal and Plant Health Inspection Service shall be conducted.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in § 113.300, except § 113.34, and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the vaccine judged accordingly.

(2) Safety test: Final container samples of completed product from each serial shall be tested to determine whether the vaccine is safe for use in susceptible young chickens. Vaccines recommended for use in chickens 10 days of age or younger shall be tested in accordance with paragraphs (d)(2)(i), (ii), and (iii) of this section.

(i) Twenty-five susceptible chickens, 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated by the eye drop method with the equivalent of 10 doses of vaccine and the chickens observed each day for 21 days. Severe respiratory signs or death shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has 3 failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and may be repeated.

(iv) Vaccines not recommended for use in chickens 10 days of age or younger shall be tested for safety as follows:

Each of twenty-five 3 to 5 week old Newcastle disease susceptible chickens shall be vaccinated as recommended on the label with the equivalent of ten doses and observed each day for 21 days. If any of the birds show severe clinical signs of disease or death during the observation period due to causes attributable to the product, the serial is unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{5.5}$ EID_{50} per dose.

[39 FR 44727, Dec. 27, 1974, as amended at 40 FR 18407, Apr. 28, 1975; 40 FR 23721, June 2, 1975; 40 FR 41090, Sept. 5, 1975; 42 FR 43618, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

EFFECTIVE DATE NOTE: At 72 FR 72564, Dec. 21, 2007, § 113.329 was amended by removing paragraph (c)(5) and redesignating paragraphs (c)(6) and (c)(7) as paragraphs (c)(5) and (c)(6), effective Jan. 22, 2008.

§ 113.330 Marek's Disease Vaccines.

Marek's disease vaccine shall be prepared from virus-bearing tissue culture cells. Only Master Seed Virus which has been established as pure, safe, and

immunogenic shall be used for preparing the production seed virus for vaccine production.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300, and the requirements prescribed in this section. The identity test required in §113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Each lot of Master Seed Virus shall also be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(b) *Safety test.* The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(1) Specific pathogen free chickens or embryos, negative for Marek's disease virus antibodies, and from the same source, shall be isolated into the following groups:

(i) *Group 1.* At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) *Group 2.* At least 50 test subjects shall be injected with a very virulent Marek's disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek's disease in at least 80 per cent of the chickens within 50 days.

(iii) *Group 3.* Fifty uninoculated controls. For *in ovo* studies, this group should receive a sham inoculation of diluent.

(iv) *Group 4.* For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(2) At least 40 chickens in each group shall survive to 5 days of age. All chickens that die shall be necropsied and examined for lesions of Marek's disease and cause of death. The test shall be judged according to the following criteria:

(i) At 50 days of age, the remaining chickens in group 2 shall be killed and examined for gross lesions of Marek's disease. If at least 80 percent of this group do not develop Marek's disease, the test is inconclusive and may be repeated.

(ii) At 120 days of age, the remaining chickens in groups 1, 3, and 4 shall be weighed, killed, and necropsied. If less than 30 of the chickens in group 3 survive the 120 day period, or if any of the chickens in group 3 have gross lesions of Marek's disease at necropsy, the test is declared inconclusive. If less than 30 chickens in groups 1 and 4 survive the 120 day period; or if any of the chickens in groups 1 and 4 have gross lesions of Marek's disease at necropsy; or if the average body weight of the chickens in groups 1 or 4 is significantly (statistically) different from the average in group 3 at the end of the 120 days, the lot of Master Seed Virus is unsatisfactory.

(3) For tests involving *in ovo* inoculation, hatchability results shall also be reported for each group.

(c) *Immunogenicity.* Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity at the highest passage level allowed for the product, and the virus dose to be used shall be established as follows:

(1) Specific pathogen free chickens or embryos, negative for Marek's disease antibodies, and from the same source, shall be isolated into the following groups:

(i) *Group 1.* A minimum of 35 test subjects shall be inoculated with the vaccine, using the recommended route, at 1 day of age for chicks or 18 days of embryonation for embryos. The dose used shall be established by 5 replicate virus titrations conducted by a cell culture system or other titration method acceptable to APHIS.

(ii) *Group 2.* A minimum of 35 nonvaccinated test subjects shall be held as challenge controls.

(iii) *Group 3.* A minimum of 25 nonvaccinated test subjects shall be held as nonchallenge controls.

(iv) *Group 4.* Except for studies evaluating vaccines which contain only a Serotype 3 virus as the Marek's disease fraction, a minimum of 35 chicks shall be vaccinated at 1 day of age with a licensed Serotype 3 vaccine, in order to document the severity of the very virulent challenge.

(2) At least 30 chickens in groups 1, 2, and 4, and at least 20 chickens in group 3, shall survive to 5 days of age. All chickens in groups 1, 2, and 4 shall be challenged at 5 days of age in the following manner:

(i) For studies evaluating vaccines which contain only a Serotype 3 virus as the Marek's disease fraction, groups 1 and 2 shall be inoculated with a standard virulent challenge virus provided or approved by APHIS.

(ii) For all other Marek's disease vaccines, groups 1, 2, and 4 shall be inoculated with a very virulent challenge virus provided or approved by APHIS.

(3) All chickens shall be observed until 7 weeks of age, necropsied, and examined for grossly observable lesions consistent with Marek's disease. All chickens dying before the end of the 7 week observation period shall be necropsied and evaluated for gross lesions of Marek's disease. Any chickens not so examined shall be scored as positive for Marek's disease.

(4) For a valid test, at least 80 percent of the chickens in group 2 must develop grossly observable lesions, none of the chickens in group 3 shall develop grossly observable lesions, and (when included) greater than 20 percent of the chickens in group 4 must develop grossly observable lesions.

(5) For a valid test to be considered satisfactory, at least 80 percent of the chickens in group 1 must remain free of grossly observable lesions. The appropriate product claim resulting from a satisfactory test would be to aid in the prevention of Marek's disease, for vaccines containing only a Serotype 3 virus as the Marek's disease fraction, or to aid in the prevention of very virulent Marek's disease, for all other vaccines.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable requirements prescribed in § 113.300. The identity test required in § 113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Final container samples of completed product shall also meet the requirements in paragraphs (d) (1), (2), and (3) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test.* The chicken embryo inoculation test prescribed in § 113.37 shall be conducted, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(2) *Safety test.* At least 25 one-day-old, specific pathogen free chickens shall be injected, by the subcutaneous route, with the equivalent of 10 chicken doses of virus (vaccine concentrated 10X). The chickens shall be observed each day for 21 days. Chickens dying during the period shall be examined, cause of death determined, and the results recorded.

(i) If at least 20 chickens do not survive the observation period, the test is inconclusive.

(ii) If lesions of any disease or cause of death are directly attributable to the vaccine, the serial is unsatisfactory.

(iii) If less than 20 chicks survive the observation period and there are no deaths or lesions attributable to the vaccine, the test may be repeated one time, *Provided,* that if the test is not repeated, the serial shall be declared unsatisfactory.

(3) *Potency test.* The samples shall be titrated using a cell culture system or other titration method acceptable to APHIS. For vaccines composed of more than one Marek's disease virus serotype, each fraction shall be titrated in a serotype-specific manner.

(i) Samples of desiccated vaccine shall be incubated at 37°C for 3 days before preparation for use in the potency test. Samples of desiccated or frozen vaccine shall be reconstituted in diluent according to the label recommendations, and held in an ice bath at 0°C to 4°C for 2 hours prior to use in the potency test.

(ii) For a serial or subserial to be eligible for release, each serotype contained in the vaccine shall have a virus titer per dose which is at least 3 times greater than the number of plaque forming units (pfu) used in the immunogenicity test prescribed in paragraph (c) of this section, but not less than 1000 pfu per dose.

(iii) When tested (without the pretest incubation of desiccated products) at

any time within the expiration period, each serotype contained in the vaccine shall have a virus titer per dose which is at least 2 times the number of pfu used in the immunogenicity test, but not less than 750 pfu per dose.

[61 FR 33841, July 1, 1996]

§ 113.331 Bursal Disease Vaccine.

Bursal Disease Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly. Each lot of Master Seed Virus used in the preparation of modified live virus vaccines shall also be nonpathogenic to chickens as determined by the following procedures:

(1) Each of twenty-five 1-day-old bursal disease susceptible chickens (vaccinates) shall be injected subcutaneously with 10 times the recommended dose of vaccine virus and observed for 21 days. Fifteen chickens of the same source and hatch shall be kept isolated as controls.

(i) Seventeen days postvaccination, each of five controls shall be administered at least $10^{2.0}$ EID_{50} of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. The remaining controls shall be used as negative controls.

(ii) If the vaccinates do not remain free of clinical signs of bursal disease, the Master Seed Virus is unsatisfactory. If unfavorable reactions which are not attributable to the Master Seed Virus occur in more than two of the vaccinates, the test shall be declared inconclusive and may be repeated.

(iii) Twenty-one days postvaccination, the vaccinates and the controls shall be necropsied and examined for gross lesions of bursal disease. If more than two of the vaccinates have such lesions, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls or less than four of the positive controls have such lesions, the test is inconclusive and may be repeated. For purposes of this test, gross lesions shall include obvious pathological processes and/or obvious reduction in size of the bursa from normal.

(2) Each of thirty-five 3- to 4-week-old bursal disease susceptible chickens (vaccinates) shall be vaccinated with approximately one minimum protective dose of vaccine virus as determined in paragraph (c) of this section. Each of 10 chickens of the same source and hatch shall be administered at least $10^{2.0}$ EID_{50} of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. Also, each of 20 additional chickens of the same source and hatch shall be isolated and held as negative controls.

(i) Three or four days postvaccination, 10 of the vaccinates, the 10 positive controls, and 10 of the negative controls shall be necropsied and examined for gross lesions of bursal disease. If any of the vaccinates have such lesions, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls or less than 8 of the positive controls have such lesions, the test is inconclusive and may be repeated. For purposes of this test, gross lesions shall include peri-bursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue.

(ii) Fourteen days post-vaccination, the remaining vaccinates and negative controls shall be necropsied and examined for obvious bursal atrophy. If any of the vaccinates have such atrophy, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls have such atrophy, the test is inconclusive and may be repeated.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and